PHOSPHORAMIDE COMPOUNDS

Related Applications

This application is a continuation under 37 C.F.R. 1.53(b) of U.S. Application Serial No. 10/047,465 filed January 14, 2002, which is a continuation under 35 USC 111(a) of International Application No. PCT/US00/19361 filed July 14, 2000 and published in English as WO 01/04130 A1 on January 18, 2001, which claims priority from U.S. Provisional Application Serial No. 60/143,799 filed July 14, 1999, which applications and publication are incorporated herein by reference.

United States Government Funding

The invention described herein was made with government support under Grant Number CA34619 awarded by the National Cancer Institute, and under Grant Number GM08298 awarded by the National Institutes of Health - National Institute of General Medical Sciences Predoctoral Training Grant in Chemical Pharmacology. The United States Government has certain rights in the invention.

Background of the Invention

Increased selectivity of anti-cancer agents is an important factor in designing new drugs for the treatment of cancer. The design and synthesis of novel compounds that can be activated selectively in cancer cells is therefore an attractive way to target the inhibition of tumor growth. This strategy ensures that cytotoxicity occurs selectively in malignant cells and might lead to a reduction of many of the side effects commonly caused by chemotherapeutic agents currently in use. An approach to the enhancement of selectivity for cytotoxic chemotherapy involves the design of prodrugs that undergo preferential activation by enzymes that are overexpressed in tumors. These prodrugs, which are not cytotoxic until they are metabolically activated, can serve to deliver selectively the cytotoxic agent to the tumor site.

One such prodrug that is used clinically is the compound cyclophosphamide (**1**, figure 1). Cyclophosphamide is activated to 4-

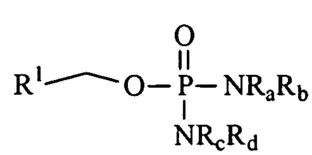
hydroxycyclophosphamide (**1a**) by hepatic cytochrome P-450 oxidation (figure 1). Subsequent β-elimination from the aldehyde tautomer of **1a** releases phosphoramide mustard **2** as the active drug which can cyclize intramolecularly to a short-lived electrophilic aziridinium ion intermediate (**3**). Nucleophilic addition can then occur and the cyclization/addition process can be repeated. Ultimately, if DNA is the nucleophile, the phosphoramide mustard can cross-link DNA and inhibit further DNA replication, a process that leads to cell death.

The design of chemotherapeutic quinone prodrugs that are bioreductively activated by the enzyme DT-diaphorase has also been investigated (see for example P. Workman, *Oncol. Res.*, **1994**, *6*, 461-475; and R.J. Riley and P. Workman, *Biochem. Pharmacol.*, **1992**, *43*, 1657-1669). Many of the compounds studied include benzimidazolequinone, benzoquinone and naphthoquinone prodrugs, with and without an alkylating moiety attached to the core ring structure, and indolequinone analogs patterned after the known cytotoxic agents Mitomycin C and E09. Sartorelli et al prepared a series of naphthoquinone prodrugs that could potentially be transformed into alkylating moieties following the expulsion of a leaving group from the bioreductively activated compound (see for example, N.E. Sladek, *Pharmac. Ther.*, **1988**, *37*, 301-355; and M. Colvin et al., *Cancer Res.*, **1976**, *36*, 1121-1126).

Despite the reported success in treating cancer with the compound Cyclophosphamide, there is currently a need for structurally novel therapeutic agents that can be used to treat cancer.

Summary of the Invention

Applicant has discovered a series of novel compounds that possess useful cytotoxic properties when administered *in vivo*. Accordingly, the invention provides a compound of formula I:



(I)

wherein:

R^1 is an organic releasing group comprising a quinone ring;

R_a, R_b, R_c, and R_d are each independently hydrogen, $(C_1\text{-}C_6)$alkyl, or $-CH_2CH_2X$; and

each X is independently halo, $(C_1\text{-}C_6)$alkylsulfonyl, halo$(C_1\text{-}C_6)$alkylsulfonyl, or arylsulfonyl, wherein each aryl is optionally substituted with one or more (e.g. 1, 2, 3, or 4) halo, $(C_1\text{-}C_6)$alkyl, halo$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_1\text{-}C_6)$alkoxycarbonyl, cyano, nitro, or trifluoromethoxy;

provided at least two of R_a, R_b, R_c, and R_d are $-CH_2CH_2X$;

or a pharmaceutically acceptable salt thereof.

The invention also provides a pharmaceutical composition comprising a compound of formula I, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable diluent or carrier.

Additionally, the invention provides a therapeutic method for preventing or treating cancer (e.g. a tumor) comprising administering to a mammal in need of such therapy, an effective amount of a compound of formula I, or a pharmaceutically acceptable salt thereof.

The invention also provides a compound of formula I for use in medical therapy (preferably for use in treating cancer, such as a tumor) as well as the use of a compound of formula I for the manufacture of a medicament for the treatment of cancer (e.g. a tumor) in a mammal, such as a human.

The invention also provides processes and intermediates useful for preparing compounds of formula I, or salts thereof.

Brief Description of the Figures

FIG. 1 illustrates the activation of cyclophosphamide *in vivo*.

Detailed Description

The following definitions are used, unless otherwise described: halo is fluoro, chloro, bromo, or iodo. Alkyl, alkoxy, etc., denote both straight and branched groups; but reference to an individual radical such as "propyl" embraces only the straight chain radical, a branched chain isomer such as "isopropyl" being specifically referred to. Aryl denotes a phenyl radical or an ortho-fused bicyclic carbocyclic radical having about nine to ten ring atoms in which at least one ring is aromatic.

As used herein the term "organic releasing group comprising a quinone ring" includes mono-, bi- and poly-cyclic ring systems that comprise at least one quinone ring, which ring systems are capable of releasing a group of formula (V):

$$^{-}O-\underset{\displaystyle NR_cR_d}{\overset{\displaystyle O}{\overset{\|}{\underset{|}{P}}}}-NR_aR_b \qquad \text{(V)}$$

from a compound of formula I when the compound of formula I is administered to a mammal (e.g. a human). Preferred releasing groups include cyclic ring systems that can be reduced *in vitro* by the enzyme DT-diaphorase, leading to the release of the group of formula (V). Other preferred releasing groups include cyclic ring systems that can be substituted *in vitro* by glutathione (or other nucleophyles), leading to the release of the group of formula (V). A more preferred releasing groups is a group of formula II, III, or IV as described herein.

As used herein the term preventing or treating cancer includes killing cancer cells and/or inhibiting their growth or proliferation.

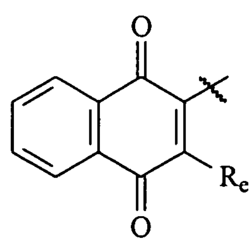
It will be appreciated by those skilled in the art that compounds of the invention having a chiral center may exist in and be isolated in optically active and racemic forms. Some compounds may exhibit polymorphism. It is to be

understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which possess the useful properties described herein, it being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase) and how to determine anti-cancer activity using the standard tests described herein, or using other similar tests which are well known in the art.

Specific and preferred values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents.

Specifically, $(C_1\text{-}C_6)$alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl, pentyl, 3-pentyl, or hexyl; $(C_1\text{-}C_6)$alkoxy can be methoxy, ethoxy, propoxy, isopropoxy, butoxy, iso-butoxy, sec-butoxy, pentoxy, 3-pentoxy, or hexyloxy; $(C_1\text{-}C_6)$alkanoyl can be acetyl, propanoyl or butanoyl; halo$(C_1\text{-}C_6)$alkyl can be iodomethyl, bromomethyl, chloromethyl, fluoromethyl, trifluoromethyl, 2-chloroethyl, 2-fluoroethyl, 2,2,2-trifluoroethyl, or pentafluoroethyl; $(C_1\text{-}C_6)$alkoxycarbonyl can be methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, pentoxycarbonyl, or hexyloxycarbonyl; $(C_1\text{-}C_6)$alkylthio can be methylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, pentylthio, or hexylthio; and $(C_1\text{-}C_6)$alkanoyloxy can be formyloxy, acetoxy, propanoyloxy, butanoyloxy, isobutanoyloxy, pentanoyloxy, or hexanoyloxy.

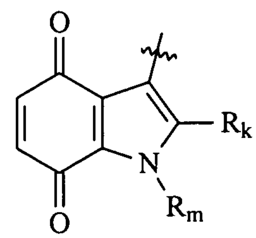
A specific value for R^1 is a group of the formula (II):



(II)

wherein R_e is hydrogen, halo, $(C_1\text{-}C_6)$alkyl, halo$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkanoyloxy, cyano, nitro, or $(C_1\text{-}C_6)$alkylthio;
and wherein the benz ring is optionally substituted by one or more (e.g. 1, 2, 3, or 4) hydroxy, halo, $(C_1\text{-}C_6)$alkyl, halo$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkylthio; $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_1\text{-}C_6)$alkoxycarbonyl, cyano, nitro, mercapto, trifluoromethoxy, or NR_fR_g; wherein each R_f and R_g is independently hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkanoyl, phenyl, benzyl, or phenethyl; or R_f and R_g together with the nitrogen to which they are attached are pyrrolidino, piperidino or morpholino.

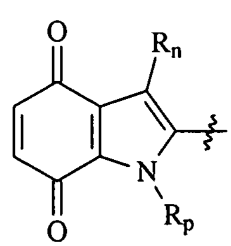
Another specific value for R^1 is a group of the formula (III):



(III)

wherein R_k is hydrogen or $(C_1\text{-}C_6)$alkyl; R_m is hydrogen or $(C_1\text{-}C_6)$alkyl, phenyl, benzyl, or phenethyl; and wherein the benz ring is optionally substituted by one or two hydroxy, halo, $(C_1\text{-}C_6)$alkyl, halo$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkylthio; $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_1\text{-}C_6)$alkoxycarbonyl, cyano, nitro, mercapto, trifluoromethoxy, or NR_fR_g; wherein each R_f and R_g is independently hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkanoyl, phenyl, benzyl, or phenethyl; or R_f and R_g together with the nitrogen to which they are attached are pyrrolidino, piperidino or morpholino.

Another specific value for R^1 is a group of the formula (IV):



(IV)

wherein R_n is hydrogen or $(C_1\text{-}C_6)$alkyl; R_p is hydrogen or $(C_1\text{-}C_6)$alkyl, phenyl, benzyl, or phenethyl; and wherein the benz ring is optionally substituted by one or two hydroxy, halo, $(C_1\text{-}C_6)$alkyl, halo$(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkoxy, $(C_1\text{-}C_6)$alkylthio; $(C_1\text{-}C_6)$alkanoyl, $(C_1\text{-}C_6)$alkanoyloxy, $(C_1\text{-}C_6)$alkoxycarbonyl, cyano, nitro, mercapto, trifluoromethoxy, or NR_fR_g; wherein each R_f and R_g is independently hydrogen, $(C_1\text{-}C_6)$alkyl, $(C_1\text{-}C_6)$alkanoyl, phenyl, benzyl, or phenethyl; or R_f and R_g together with the nitrogen to which they are attached are pyrrolidino, piperidino or morpholino.

A specific value for X is bromo, chloro, mesyl, trifluoromethylsulfonyl, or tosyl. A more specific value for X is bromo.

A specific value for R_e is hydrogen, halo, methyl, or methylthio.

A specific value for R_h is hydrogen or methyl.

A specific value for R_k is hydrogen or methyl.

A specific value for R_m is hydrogen or methyl.

A specific value for R_n is hydrogen or methyl.

A specific value for R_p is hydrogen or methyl.

A specific group of compounds of formula I are compounds wherein R_a is $(C_1\text{-}C_6)$alkyl.

A specific group of compounds of formula I are compounds wherein R_c is $(C_1\text{-}C_6)$alkyl.

A specific group of compounds of formula I are compounds wherein R_a and R_b are each independently $-CH_2CH_2X$.

A specific group of compounds of formula I are compounds wherein R_c, and R_d are each independently $-CH_2CH_2X$.

A specific group of compounds of formula I are compounds wherein R_b and R_d are each independently $-CH_2CH_2X$.

A more specific group of compounds of formula I are compounds wherein R_a is methyl.

A more specific group of compounds of formula I are compounds wherein R_c is methyl.

A more specific group of compounds of formula I are compounds wherein R_a and R_b are each independently $-CH_2CH_2Br$.

A more specific group of compounds of formula I are compounds wherein R_c, and R_d are each independently $-CH_2CH_2Br$.

A more specific group of compounds of formula I are compounds wherein R_b and R_d are each independently $-CH_2CH_2Br$.

A more specific group of compounds of formula I are compounds wherein R_a and R_b are each independently $-CH_2CH_2Cl$.

A more specific group of compounds of formula I are compounds wherein R_c, and R_d are each independently $-CH_2CH_2Cl$.

A more specific group of compounds of formula I are compounds wherein R_b and R_d are each independently $-CH_2CH_2Cl$.

A preferred compound of the invention is:

2-(1,4-naphthoquinonyl)methyl *N,N*-bis(2-chloroethyl) phosphorodiamidate;

2-(3-Methyl-1,4-naphthoquinonyl)methyl *N,N*-bis(2-chloroethyl) phosphorodiamidate;

2-(3-Thiomethyl-1,4-naphthoquinonyl)methyl *N,N*-bis(2-chloroethyl) phosphorodiamidate;

2-(3-Bromo-1,4-naphthoquinonyl)methyl *N,N*-bis(2-chloroethyl) phosphorodiamidate;

2-(1,4-Naphthoquinonyl)methyl *N,N*-bis(2-bromoethyl) phosphorodiamidate;

2-(3-Methyl-1,4-naphthoquinonyl)methyl *N,N*-bis(2-bromoethyl) phosphorodiamidate;

2-(1,4-Naphthoquinonyl)methyl bis[*N*-(2-chloroethyl)] phosphorodiamidate;

2-(1,4-Naphthoquinonyl)methyl bis[*N*-methyl-*N*-(2-bromoethyl)]phosphorodiamidate;

2-(3-Methyl-1,4-naphthoquinonyl)methyl bis[*N*-methyl-*N*-(2-bromoethyl)] phosphorodiamidate;

2-(1,4-Naphthoquinonyl)methyl bis[*N*-methyl-*N*-(2-chloroethyl)] phosphorodiamidate;

3-(5-Methoxy-1-methyl-4,7-indolequinonyl)-methyl bis[*N*-methyl-*N*-(2-bromoethyl)] phosphorodiamidate;

3-(5-Methoxy-1-methyl-4,7-indolequinonyl)methyl N,N-bis(2-bromoethyl)-phosphorodiamidate;

2-(5-Methoxy-1-methyl-4,7-indolequinonyl)methyl bis[N-methyl-N-(2-bromoethyl)]phosphorodiamidate;

2-(5-Methoxy-1-methyl-4,7-indolequinonyl)methyl N,N-bis(2-chloroethyl)-phosphorodiamidate; or

2-(5-Methoxy-1-methyl-4,7-indolequinonyl)methyl N,N-bis(2-bromoethyl)-phosphorodiamidate;

or a pharmaceutically acceptable salt thereof.

A more prefered compound of the invention is:

3-(5-Methoxy-1-methyl-4,7-indolequinonyl)methyl N,N-bis(2-bromoethyl)-phosphorodiamidate;

2-(5-Methoxy-1-methyl-4,7-indolequinonyl)methyl bis[N-methyl-N-(2-bromoethyl)]phosphorodiamidate;

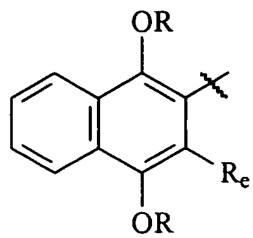
2-(5-Methoxy-1-methyl-4,7-indolequinonyl)methyl N,N-bis(2-chloroethyl)-phosphorodiamidate; or

2-(5-Methoxy-1-methyl-4,7-indolequinonyl)methyl N,N-bis(2-bromoethyl)-phosphorodiamidate;

or a pharmaceutically acceptable salt thereof.

Processes for preparing compounds of formula I are provided as further embodiments of the invention and are illustrated by the following procedures in which the meanings of the generic radicals are as given above unless otherwise qualified.

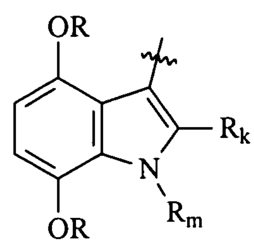
A compound of formula I wherein R^1 is a group of formula II can be prepared by oxidizing a corresponding compound wherein R^1 is a group of formula VI:



(VI)

wherein each R is independently (C_1-C_6)alkyl. The oxidation can be carried out under standard conditions, for example using ceric ammonium nitrate. Fremy's salt, or silver (II) oxide. Suitable conditions for carrying out such an oxidation are described in the Examples hereinbelow.

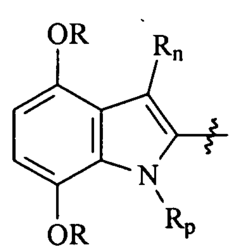
A compound of formula I wherein R^1 is a group of formula III can be prepared by oxidizing a corresponding compound wherein R^1 is a group of formula VII:



(VII)

wherein each R is independently (C_1-C_6)alkyl. The oxidation can be carried out under standard conditions, for example using ceric ammonium nitrate, Fremy's salt, or silver (II) oxide. Suitable conditions for carrying out such an oxidation are described in the Examples hereinbelow.

A compound of formula I wherein R^1 is a group of formula IV can be prepared by oxidizing a corresponding compound wherein R^1 is a group of formula VIII:



(VIII)

wherein each R is independently (C_1-C_6)alkyl. The oxidation can be carried out under standard conditions, for example using ceric ammonium nitrate, Fremy's

salt, or silver (II) oxide. Suitable conditions for carrying out such an oxidation are described in the Examples hereinbelow.

An intermediate useful for preparing a compound of formula I is a corresponding compound of formula I wherein R^1 is a group of formula VI, VII, or VIII.

In cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compounds as salts may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartrate, succinate, benzoate, ascorbate, α-ketoglutarate, and α-glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

The compounds of formula I can be formulated as pharmaceutical compositions and administered to a mammalian host, such as a human patient in a variety of forms adapted to the chosen route of administration, i.e., orally or parenterally, by intravenous, intramuscular, topical or subcutaneous routes.

Thus, the present compounds may be systemically administered, e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The

percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in

liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

For topical administration, the present compounds may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent

pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to deliver the compounds of formula I to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the compounds of formula I can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949.

Generally, the concentration of the compound(s) of formula I in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

In general, however, a suitable dose will be in the range of from about 0.5 to about 100 mg/kg, e.g., from about 10 to about 75 mg/kg of body weight per day, such as 3 to about 50 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 mg/kg/day, most preferably in the range of 15 to 60 mg/kg/day.

The compound is conveniently administered in unit dosage form; for example, containing 5 to 1000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of active ingredient per unit dosage form.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye.

Determination of releasing group activity

To determine whether representative compounds of formula I deliver the alkylating moiety (V) following quinone reduction, representative compounds of the invention were chemically reduced and the reaction was monitored by ^{31}P NMR. The quinone was dissolved in CH_3CN (0.3 mL, or THF for **16a**) and the activating agent (glutathione, or sodium dithionite) was dissolved in cacodylate buffer (0.4 mL, 0.4 M, pH 7.7). The buffer solution was added to the organic solution and the pH of the mixture was adjusted to ~7.4. The reaction mixture was transferred to a 5-mm NMR tube, and the data acquisition was started (pulse delay 30 μs). Spectra were taken every 2.5 minutes for 0.5 h, then every 5 min for 0.5 h, then every 10 min for 1 h, and time points for each spectrum were assigned from the initiation of the reaction. Chemical shifts are reported relative to the TPPO reference. The temperature of the probe was maintained at 37 °C, if necessary, using the Bruker variable temperature unit. The relative concentrations of the intermediates were determined by measuring the peak areas.

Naphthoquinone **15a** was reduced with sodium dithionite (3 equiv, 3:4 CH_3CN:0.4 M cacodylate buffer, pH ~ 7.4, 37 °C), and the reaction was followed by ^{31}P NMR. The resonance for quinone 3c at −5.3 ppm disappeared within 5 minutes and was replaced by the resonance for the corresponding phosphoramide mustard at −12.2 ppm. Similar results were obtained for compounds **16a, 17,** and **40a**.

Other Cellular Mechanisms of Quinone Activation

The Michael addition of sulfur nucleophiles to naphthoquinones is well known; and addition to the 3-position of the quinone might provide another pathway for phosphorodiamidate anion release. Two possible products could be predicted from the Michael addition of a nucleophile to the naphthoquinone. Addition at the 2-position leads to reversible formation of the kinetic product; and addition at the 3-position provides an intermediate that could expel the nucleophile in a reversible reaction, or could expel the phosphorodiamidate anion in an irreversible step. Experiments were carried out using sodium dimethyldithiocarbamate (DDTC), which contains a highly nucleophilic sulfur that is anionic at physiologic pH. Activation of **16a** with DDTC (3 equiv, 3:4 THF:0.4 M cacodylate buffer, pH ~ 7.3, RT) was monitored using ^{31}P NMR. Three equivalents of nucleophile were used, assuming that one equivalent would be consumed by the Michael addition and the other two would be consumed by reaction with the resulting phosphorodiamidate anion. The resonance for quinone **16a** (-5.7 ppm) disappeared and was replaced by the resonance for the phosphorodiamidate anion at –13.1 ppm within 5 min after addition of DDTC, confirming that nucleophilic activation of the naphthoquinone is rapid and complete.

The experiment above demonstrate that compound **16a** is activated by a potent sulfur nucleophile. Thus, the following study was conducted to determine whether glutathione would activate compounds of formula I in a similar way. Glutathione, the primary non-protein intracellular thiol, is responsible for maintaining the cellular redox environment and removing potential electrophilic cytotoxins. It is overproduced (1 – 10 mM intracellular concentrations) in many cancer cell lines, particularly those that have acquired resistance to anticancer drugs. Thus, the reaction of **15a** with glutathione (3 equiv, 1:1.3 CH_3CN:0.4 M cacodylate buffer, pH ~ 7.5, RT) was monitored by ^{31}P NMR. The reaction with glutathione was essentially identical to that of DDTC; the resonance for quinone **15a** had disappeared and was replaced by the resonance for phosphorodiamidate anion within 4 minutes of glutathione addition. The result was essentially identical when only one equivalent of glutathione was used, again suggesting

that activation of this compound is complete before cyclization of the phosphorodiamidate anion can occur.

The cytotoxic properties of a compound of the invention can be determined using *in vitro* pharmacological models which are well known to the art, or can be determined using Test A described below.

Test A: *In Vitro* Cytotoxicity (Clonogenic Survival of HT-29 and BE cells):

The human colon carcinoma cell line HT-29 was obtained from the American Type Culture Collection (ATCC), and the BE cell line was provided by Dr. D. Ross, University of Colorado. A modification of the procedure described by Miribelli et al *Cancer Research*, **1985**, *45*, 32-39 was used to determine the clonogenic survival of the cells. HT-29 and BE cells in exponential growth were suspended in unsupplemented Eagles MEM medium (10 mL) at a final density of 1.7 - 2 x 10^5 cells/mL. Unsupplemented medium contains Minimum Essential Medium (Gibco) and HEPES (0.02 M). The drug stock solutions (0.1 – 40 mM) were prepared using either ethanol or dimethyl sulfoxide as solvent. The maximum amount of DMSO or ethanol used in the drug treatments was 1% of the total volume. Appropriate volumes (6.5 to 100 μL) of the drug stock solution were added to five vials of the cell suspensions, to give five different final drug concentrations, and 100 μL of solvent was added to a sixth vial for a control. The treated cells were incubated for 2 h (37°C, 5% CO_2). The cells were spun down and rinsed three times with supplemented medium (3 mL) and then diluted in 5 mL of supplemented medium. Supplemented medium is prepared by adding Fetal Bovine Serum (10%), gentamicin (0.05 mg / mL), L-glutamine (0.03 mg / mL), and sodium pyruvate (0.1 mM) to unsupplemented medium. The cells were counted using a Coulter Counter and then plated at 2-3 different densities for each drug concentration and incubated for 10 days. The colonies were stained with 0.5% crystal violet in 95% ethanol and those colonies comprised of 50 or more cells were counted using a microscope and pen-style counter. The LC_{99} of each compound (the concentration at which there is a 1% cell survival) was determined by plotting

the log surviving fraction vs. drug concentration. Data for representative compounds of the invention is provided in Table 1.

Table 1

Compound	HT-29 LC_{99} (μM)	BE LC_{99} (μM)
15a	11	13
15b	2.1	1.0
15c	4.2	5.9
15d	4.1	3.3
16a	7.8	4.6
16b	1.7	1.2
17	14	7.8
40a	4.5	1.2
40b	1.5	0.44
40c	4.5	2.8
47	0.07	0.14

The above data suggests that compounds of formula I wherein R_a and/or R_c are alkyl are unexpectedly more cytotoxic than the corresponding compounds wherein R_a and R_c are hydrogen. Thus, prefered compounds of the invention include compounds of formula I wherein R_a and/or R_c are alkyl.

The growth inhibitory properties of representative compounds of the invention were also determined by evaluating their growth inhibitory activity against a series of human tumor cell lines using a 72-h drug exposure. Cell counts were measured using the MTT assay. The results are summarized in Table 2.

Table 2. Growth inhibitory activity of compounds **1-6**

	IC$_{50}$, nM[a]							
Cpd	**IGROV**	**MDA231**	**PC-3**	**HT-29**	**PaCa-2**	**A498**	**A549**	**UMUC3**
40a	2048	3228	2559	5925	2303	4459	11181	9953
16a	18932	14656	9193	15266	4417	15599	4583	2823
47	494	40	66	65	25	344	23	2
4		194	649	241	215	998	398	10
5	7588	210	420	93	13	2650	260	14
6a	1700	3500	170	340	6	862	203	22
6b	1473	1628	195	61	35	199	9	1

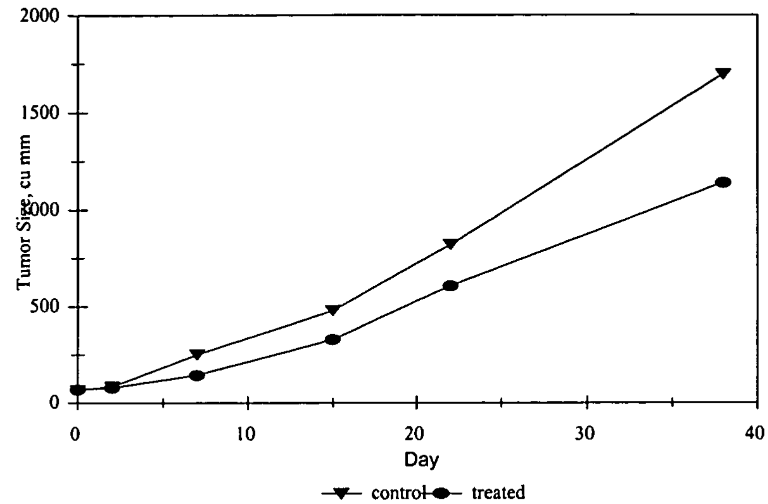
[a]IGROV=ovarian, MDA231=breast, PC-3=prostate, HT-29=colon, PaCa-2=pancreas, A498=kidney, A549=lung, UMUC3=bladder

Several interesting conclusions are apparent from these data. Compounds **40a** and **16a** are moderately active (IC$_{50}$ in the low micromolar range) against all cell lines and show relatively little selectivity for tumor cells of different tissue types. Second, compounds **3**, **5**, **6a** and **6b** show significant selectivity and exceptional potency against PaCa-2 and UMUC3 cell lines, with IC$_{50}$ values in the low nanomolar range. These results are especially significant because pancreatic cancer and bladder cancer are difficult tumors to treat clinically. These tumors are poorly responsive to most existing anticancer drugs, and therapeutic options are very limited.

The cytotoxic properties of a compound of the invention can also be determined using *in vivo* pharmacological models which are well known to the art. For example, compound **16a** was tested in a xenograft model. This assay consisted of injecting nude mice subcutaneously with human tumor cells. The tumors were allowed to grow until a measurable mass was present (22 days), and a single dose of **16a** was administered subcutaneously. The tumor size was measured periodically and the growth inhibition of the tumor was determined. The A498$_2$LM cell line, a sub-line of the A498 human kidney carcinoma, was used for the xenograft assay. The A498$_2$LM sub-line, which has a high DT-diaphorase level (1010 nmol cytochrome c reduced/ min/mg protein), was developed by implanting the A498 cell line in mice and collecting the lung

metastases that formed from the original tumor. Compound **16a** exhibited a 40% growth inhibition on Day 38 after a single dose of 25 mg/kg (Graph 2).

Graph 2: Tumor Response to 16a



The invention will now be illustrated by the following non-limiting Examples wherein unless otherwise noted the following general procedures were followed.

General Procedures

^{1}H NMR spectra were measured on a 250 MHz Bruker NMR system equipped with a multinuclear (^{1}H, ^{13}C, ^{19}F and ^{31}P) 5-mm probe. The NMR data acquisition / processing program MacNMR was used with the Tecmag data acquisition system. ^{1}H Chemical shifts are reported in parts per million from tetramethylsilane. ^{31}P NMR spectra were obtained on the same instrument using broadband gated decoupling. Chemical shifts are reported in parts per million from a coaxial insert containing 5% phosphoric acid in H_2O. All variable temperature experiments were conducted using a Bruker variable temperature unit.

Analtech precoated silica gel glass plates (250 microns) were used to perform thin layer chromatography. The plates were visualized using UV and/or one of the following two stains: 3% phosphomolybdic acid in methanol followed by heating or 1% 4-(*p*-nitrobenzyl)pyridine in acetone followed by heating and

treatment with 3% KOH in acetone (to detect for an alkylating moiety). Chromatographic purifications were carried out by flash chromatography using silica gel grade 60 (230-400 mesh, 60 A). High performance liquid chromatography (HPLC) analyses were performed using a Beckman System Gold with a 126 Solvent Module, a 168 Detector set to either 250 or 280 nm and an Econosphere C18 5-micron column (250 mm, Alltech Associates). The mobile phase was acetonitrile : 0.1% trifluoroacetic acid in H_2O using the percentages indicated and a flow rate of 1 mL / min.

Elemental analyses were performed by the Purdue University Microanalysis Lab, West Lafayette, IN. Mass spectral data was obtained from the Purdue University Mass Spectrometry Service, West Lafayette, IN, using fast atom bombardment (FAB) with a 3-nitrobenzyl alcohol matrix.

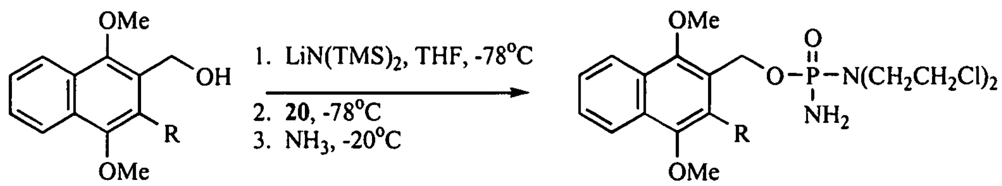
A glass-calomel electrode on either a Radiometer pH meter or an Orion PerpHect LogR meter, model 330, was used for acidity measurements. Melting points were determined on a Mel-Temp II apparatus and are uncorrected. IR spectra were recorded on a Nicolet Magna IR 550 spectrometer using either a thin film or Nujol suspension, as noted, between NaCl plates. All anhydrous reactions were carried out in either a flame dried or oven dried flask under argon. Organic solutions were concentrated on a Buchi rotary evaporator.

Chemical reagents were purchased from Aldrich except NADH, NADPH, dicumarol and cytochrome c (Sigma) and ammonia (Matheson Gas). All cell culture reagents were purchased from Gibco Life Technologies. Purified human DT-diaphorase was supplied by Dr. S. Chen, City of Hope Medical Center, CA.

Tetrahydrofuran was distilled from sodium, with benzophenone ketyl as indicator, prior to use. Methylene chloride, diisopropylethylamine, triethylamine and acetonitrile were distilled from calcium hydride prior to use.

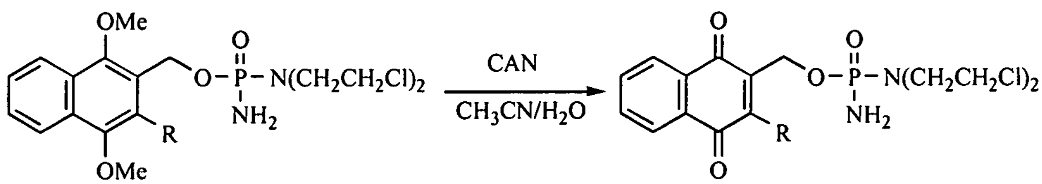
Butyllithium was purchased as a 2.5 M solution in hexanes, t-butyl hydroperoxide as a 5-6 M solution in decane, phosphorus trichloride as a 2 M solution in methylene chloride, lithium aluminum hydride as a 1.0 M solution in ether, sodium hydride as a 60% dispersion in mineral oil and lithium bis(trimethylsilyl)amide as a 1.0 M solution in tetrahydrofuran.

Schemes For Examples 1-4



23a: R = H
23b: R = CH_3
23c: R = SCH_3
23d: R = Br

25a: R = H
25b: R = CH_3
25c: R = SCH_3
25d: R = Br



25a: R = H
25b: R = CH_3
25c: R = SCH_3

15a: R = H
15b: R = CH_3
15c: R = SCH_3

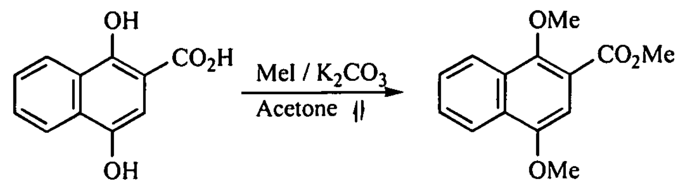
Example 1. 2-(1,4-Naphthoquinonyl)methyl *N,N*-bis(2-chloroethyl) phosphorodiamidate (**15a**)

Ceric ammonium nitrate (1.56 g, 2.85 mmol) in H_2O (10 mL) was added in portions over 15 min to a solution of **25a** (480 mg, 1.14 mmol) in acetonitrile (30 mL). The solution was stirred at room temperature for 1 h and extracted with $CHCl_3$ (3x). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (6:94 MeOH:$CHCl_3$) afforded **15a** (360 mg, 81%) as a yellow solid; R_f = 0.45 (6:94 MeOH:$CHCl_3$); mp = 105 – 107 °C; ^{1}H NMR ($CDCl_3$): δ 8.09 (m, 2H), 7.77 (m, 2H), 7.03 (t, 1H), 5.05 (m, 2H, $J_{P\text{-}H}$ = 7.1 Hz), 3.68 (t, 4H), 3.54 (m, 4H), 3.01 (bs, 2H); ^{31}P NMR ($CDCl_3$): δ 16.42

IR (Nujol): 1664, 1630, 1589 cm^{-1}. Anal. Calcd. for $C_{15}H_{17}Cl_2N_2O_4P$: C, 46.06; H, 4.38; N, 7.16. Found: C, 45.72; H, 4.17; N, 7.27.

The intermediate compound **25a** was prepared as follows.

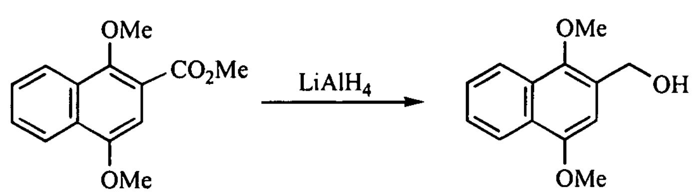
a. Methyl 1,4-dimethoxy-2-naphthoate (**22**)



22

Potassium carbonate (30.50 g, 0.22 mol) and MeI (27.5 mL, 0.44 mol) were added to a solution of 1,4-dihydroxy-2-naphthoic acid (6.00 g, 0.029 mol) in acetone (120 mL) under argon. The mixture was refluxed for 48 h. Water (50 mL) was added and the mixture was extracted with CH_2Cl_2 (5x). The combined organic layers were washed with H_2O (2x), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (15:85 EtOAc:hexanes) afforded **22** (7.05 g, 97%) as a green solid; $R_f = 0.44$ (15:85 EtOAc:hexanes); mp = 48 – 50°C; lit mp = 52 – 55°C[25]; ^{1}H NMR ($CDCl_3$): δ 8.24 (m, 2H), 7.59 (m, 2H), 7.16 (s, 1H), 4.02 (s, 3H), 4.01 (s, 3H), 4.00 (s, 3H).

b. 1,4-Dimethoxy-2-hydroxymethylnaphthalene (**23a**)



22 **23a**

A solution of lithium aluminum hydride (29.2 mL, 29.2 mmol, 1 M in ether) in ether (30 mL) was heated to reflux under argon. A solution of ester **22** (7.18 g, 29.2 mmol) in ether (30 mL) and THF (8 mL) was added dropwise over 35 min via an addition funnel. The milky yellow mixture was refluxed for 3 h and then cooled to 0 °C. Methanol (10 mL) was added dropwise and the resulting clear

yellow solution was stirred for 1 h. Saturated NH_4Cl (20 mL) and aqueous HCl (5 mL, 10%) were added and the mixture was extracted with ether (6x). The combined organic layers were washed with saturated Na_2CO_3 (2x) and H_2O (2x), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (35:65 EtOAc:hexanes) afforded **23a** (5.82 g, 92%) as a pink solid; R_f = 0.48 (35:65 EtOAc:hexanes); mp = 69 – 70°C; lit mp = 68 – 70°C[25]; ^{1}H NMR ($CDCl_3$): δ 8.23 (dd, 1H), 8.03 (dd, 1H), 7.52 (m, 2H), 6.82 (s, 1H), 4.89 (s, 2H), 3.99 (s, 3H), 3.92 (s, 3H), 1.95 (bs, 1H).

c. 2-(1,4-Dimethoxynaphthyl)methyl *N,N*-bis(2-chloroethyl) phosphorodiamidate (**25a**). Lithium bis(trimethylsilyl)amide (2.50 mL, 2.50 mmol, 1.0 M in THF) was added dropwise via syringe to a solution of alcohol **23a** (500 mg, 2.29 mmol) in THF (10 mL) at –78°C under argon. The resulting solution was stirred for 5 min and added dropwise via syringe to a solution of bis(2-chloroethyl)phosphoramidic dichloride (**20**) (710 mg, 2.75 mmol) in THF (20 mL) at –78 °C. The reaction mixture was stirred at –78 °C for 1.5 h and then was warmed to –20°C. Gaseous ammonia was passed through the reaction mixture for 10 min. The mixture was stirred for an additional 10 min, aqueous HCl (2%, 30 mL) was added and the mixture was extracted with EtOAc (4x). The combined organic layers were washed with saturated NaCl (2x), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (2:98 MeOH:EtOAc) afforded **25a** (480 mg, 50%) as a yellow oil; R_f = 0.59 (2:98 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$): δ 8.25 (dd, 1H), 8.06 (dd, 1H), 7.54 (m, 2H), 6.85 (s, 1H), 5.33 (m, 2H, $J_{P\text{-}H}$ = 7.8 Hz), 4.01 (s, 3H), 3.94 (s, 3H), 3.65 (t, 4H), 3.48 (m, 4H), 2.77 (bs, 2H); ^{31}P NMR ($CDCl_3$): δ 15.72.

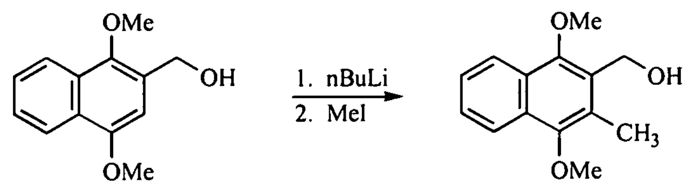
Example 2. 2-(3-Methyl-1,4-naphthoquinonyl)methyl *N,N*-bis(2-chloroethyl) phosphorodiamidate (**15b**)

Compound **15b** was prepared from **25b** (440 mg, 1.01 mmol) as described above for **15a** to give 289 mg (71%) of the product as a yellow solid

after column chromatography (3:97 MeOH:$CHCl_3$); R_f = 0.16 (3:97 MeOH:$CHCl_3$); mp = 129 – 130°C; <u>^{1}H NMR</u> ($CDCl_3$): δ 8.11 (m, 2H), 7.76 (m, 2H), 5.06 (m, 2H, J_{P-H} = 7.3 Hz), 3.66 (t, 4H), 3.47 (dt, 4H), 3.02 (bs, 2H), 2.34 (s, 3H); <u>^{31}P NMR</u> ($CDCl_3$): δ 14.27; <u>IR</u> (Nujol): 1665, 1660, 1628, 1594 cm^{-1}. Anal. Calcd. for $C_{16}H_{19}Cl_2N_2O_4P$: C, 47.43; H, 4.73; N, 6.91. Found C, 47.59; H, 4.53; N, 6.84.

The intermediate compound **25b** was prepared as follows.

a. 1,4-Dimethoxy-3-methyl-2-hydroxymethylnaphthalene (**23b**)



23a **23b**

n-Butyllithium (7.3 mL, 18.3 mmol, 2.5 M in hexanes) was added dropwise via syringe to a solution of **23a** (1.00 g, 4.58 mmol) in THF (30 mL) at –78 °C under argon. The solution was slowly warmed to room temperature and stirred for 1 h. Methyl iodide (0.34 mL, 5.50 mmol) was added dropwise and the solution was stirred for 20 min. Water (8 mL) was added and the mixture was extracted with EtOAc (4x). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (45:55 EtOAc:hexanes) afforded **23b** (0.61 g, 57%) as a yellow solid; R_f = 0.59 (45:55 EtOAc:hexanes); mp = 116 – 117°C; lit mp = 118 - 121°C[25]; <u>^{1}H NMR</u> ($CDCl_3$): δ 8.07 (dd, 2H), 7.51 (m, 2H), 4.94 (s, 2H), 3.98 (s, 3H), 3.88 (s, 3H), 2.52 (s, 3H), 1.73 (bs, 1H).

b. 2-(3-Methyl-1,4-dimethoxynaphthyl)methyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (**25b**). Compound **25b** was prepared from **23b** (500 mg, 2.15 mmol) as described above for **25a** to give 440 mg (47%) of the product as a light yellow foam after column chromatography (1:99

MeOH:EtOAc); $R_f = 0.44$ (1:99 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$): δ 8.08 (m, 2H), 7.53 (m, 2H), 5.31 (m, 2H, $J_{P-H} = 6.8$ Hz), 3.98 (s, 3H), 3.89 (s, 3H), 3.64 (t, 4H), 3.45 (dt, 4H), 2.77 (bs, 2H), 2.52 (s, 3H); ^{31}P NMR ($CDCl_3$): δ 15.65.

Example 3. 2-(3-Thiomethyl-1,4-naphthoquinonyl)methyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (**15c**)

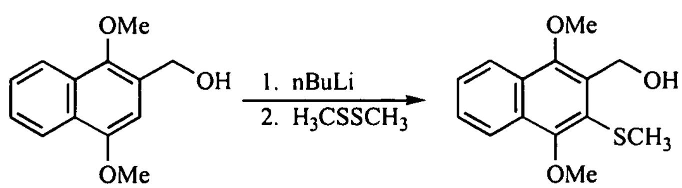
Compound **15c** was prepared from **25c** (134 mg, 0.287 mmol) as described above for **15a** to give 72.0 mg (57%) of the product as a viscous orange oil after column chromatography (2:98 MeOH:$CHCl_3$); $R_f = 0.29$ (2:98 MeOH:$CHCl_3$)

^{1}H NMR ($CDCl_3$): δ 8.10 (m, 2H), 7.75 (m, 2H), 5.21 (m, 2H, $J_{P-H} = 6.1$ Hz), 3.66 (t, 4H), 3.46 (dt, 4H), 2.97 (bs, 2H), 2.74 (s, 3H); ^{31}P NMR ($CDCl_3$): δ 15.25

IR (Nujol): 1666, 1643, 1588, 1542 cm^{-1}. Anal. Calcd. for $C_{16}H_{19}Cl_2N_2O_4PS$: C, 43.95; H, 4.38; N, 6.41. Found: C, 43.93; H, 4.21; N, 6.16.

The intermediate compound **25c** was prepared as follows.

a. 1,4-Dimethoxy-3-thiomethyl-2-hydroxymethyl-naphthalene (**23c**)



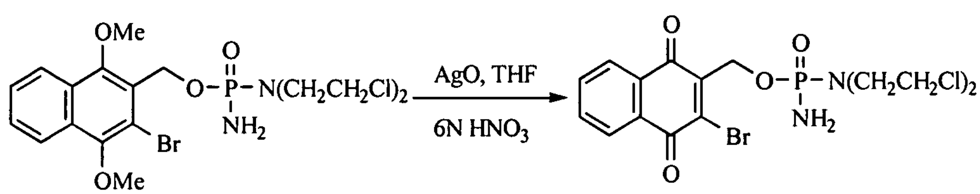
23a **23c**

n-Butyllithium (0.73 mL, 1.83 mmol, 2.5 M in hexanes) was added dropwise to a solution of **23a** (100 mg, 0.458 mmol) in THF (6 mL) at –78 °C under argon. The solution was slowly warmed to room temperature and stirred for 1 h. Dimethyl disulfide (0.050 mL, 0.55 mmol) was added dropwise. The clear, yellow solution became cloudy during the 30 min the reaction mixture was

stirred. Water (1 mL) was added and the mixture was extracted with EtOAc (3x). The combined organic layers were washed with saturated NaCl, dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (20:80 EtOAc:hexanes) afforded **23c** (71.2 mg, 59%, 82% based on recovered **23a**, 28.0 mg) as a light green oil; $R_f = 0.26$ (20:80 EtOAc:hexanes); <u>^{1}H NMR</u> ($CDCl_3$): δ 8.10 (m, 2H), 7.55 (m, 2H), 5.07 (s, 2H), 4.03 (s, 3H), 3.99 (s, 3H), 2.53 (s, 3H), 1.60 (bs, 1H).

b. 2-(3-Thiomethyl-1,4-dimethoxynaphthyl)methyl *N,N*-bis(2-chloro-ethyl) phosphorodiamidate (**25c**). Compound **25c** was prepared from **23c** (71.0 mg, 0.27 mmol) as described above for **25a** to give 76.9 mg (61%) of the product as a yellow oil after column chromatography (EtOAc); $R_f = 0.22$ (EtOAc); <u>^{1}H NMR</u> ($CDCl_3$): δ 8.12 (m, 2H), 7.57 (m, 2H), 5.48 (d, 2H, J = 4.9 Hz), 4.04 (s, 3H), 4.00 (s, 3H), 3.64 (t, 4H), 3.49 (dt, 4H), 2.73 (bs, 2H), 2.51 (s, 3H); <u>^{31}P NMR</u> ($CDCl_3$): δ 14.62.

<u>Example 4.</u> 2-(3-Bromo-1,4-naphthoquinonyl)methyl *N,N*-bis(2-chloroethyl)phosphorodiamidate (**15d**)



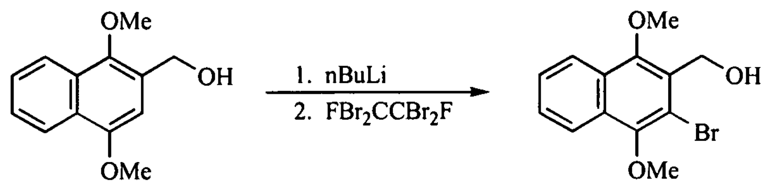
25d **15d**

Silver (II) oxide (300 mg, 2.4 mmol) was added in one portion to a solution of **25d** (200 mg, 0.40 mmol) in THF (15 mL) at room temperature. Nitric acid (6 N, 2 mL) was added to this suspension. The mixture was stirred for 20 min at room temperature, H_2O (5 mL) was added and the mixture was extracted with $CHCl_3$ (3x). The combined organic layers were washed with saturated NaCl and $NaHCO_3$ (1 M), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (2:98 MeOH:$CHCl_3$ until less polar impurities are removed, then 6:94 MeOH:$CHCl_3$) afforded **15d** (156 mg,

83%) as a yellow solid; $R_f = 0.36$ (6:94 MeOH:$CHCl_3$); mp = 134 – 135 °C; <u>^{1}H NMR</u> ($CDCl_3$): δ 8.18 (m, 2H), 7.80 (m, 2H), 5.21 (m, 2H, $J_{P\text{-}H} = 6.5$ Hz), 3.66 (t, 4H), 3.48 (dt, 4H), 2.97 (bs, 2H).
<u>^{31}P NMR</u> ($CDCl_3$): δ 15.32; IR (Nujol): 1680, 1675, 1659, 1605, 1589 cm^{-1}. Anal. Calcd. for $C_{15}H_{16}BrCl_2N_2O_4P$: C, 38.33; H, 3.43; N, 5.96. Found: C, 38.68; H, 3.42; N, 5.83.

The intermediate compound **25d** was prepared as follows.

a. 1,4-Dimethoxy-3-bromo-2-hydroxymethylnaphthalene (**23d**)



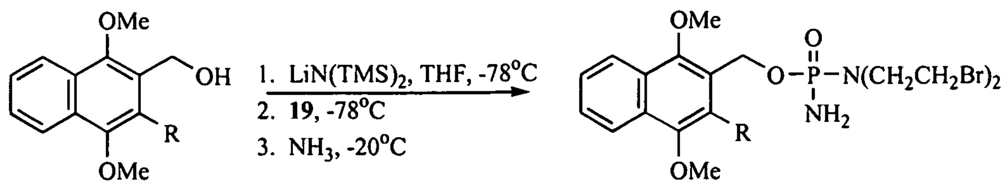
23a **23d**

n-Butyllithium (2.9 mL, 7.33 mmol, 2.5 M in hexanes) was added dropwise to a solution of **23a** (400 mg, 1.83 mmol) in THF (25 mL) at –78°C under argon. The reaction mixture was slowly warmed to room temperature and stirred for 1 h. 1,2-dibromotetrafluoroethane (0.26 mL, 2.20 mmol) was added dropwise and the solution was stirred for 1 h. Water (10 mL) was added and the mixture was extracted with EtOAc (3x). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (35:65 EtOAc:hexanes) afforded **23d** (273 mg, 50%, 63% based on recovered **23a,** 84 mg) as a yellow solid; $R_f = 0.62$ (35:65 EtOAc:hexanes); mp = 115 – 117°C; <u>^{1}H NMR</u> ($CDCl_3$): δ 8.11 (m, 2H), 7.57 (m, 2H), 5.03 (s, 2H), 4.02 (s, 3H), 3.99 (s, 3H), 2.36 (bs, 1H).

b. 2-(3-Bromo-1,4-dimethoxynaphthyl)methyl *N,N*-bis(2-chloroethyl)-phosphorodiamidate (**25d**). Compound **25d** was prepared from **23d** (270 mg, 0.91 mmol) as described above for **25a** to give 353 mg (78%) of the

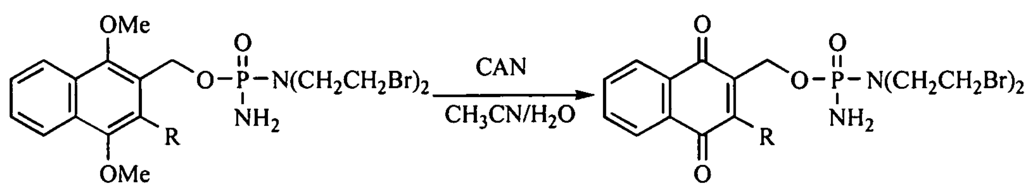
product as a white solid after column chromatography (EtOAc); $R_f = 0.17$ (EtOAc); mp = 112 – 114 °C; <u>^{1}H NMR</u> ($CDCl_3$): δ 8.12 (m, 2H), 7.60 (m, 2H), 5.40 (m, 2H, $J_{P\text{-}H} = 5.9$ Hz), 4.02 (s, 3H), 4.00 (s, 3H), 3.66 (t, 4H), 3.48 (dt, 4H), 2.82 (bs, 2H); <u>^{31}P NMR</u> ($CDCl_3$): δ 14.38.

Scheme for Examples 5 and 6



23a: R = H
23b: R = CH_3

26a: R = H
26b: R = CH_3



26a: R = H
26b: R = CH_3

16a: R = H
16b: R = CH_3

Example 5. 2-(1,4-Naphthoquinonyl)methyl *N,N*-bis(2-bromoethyl) phosphorodiamidate (**16a**)

Ceric ammonium nitrate (1.83 g, 3.33 mmol) in H_2O (15 mL) was added in portions over 15 min to a solution of **26a** (680 mg, 1.33 mmol) in CH_3CN (60 mL). The reaction was stirred at room temperature for 1 h and extracted with $CHCl_3$ (3x). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (2:98 MeOH:EtOAc) afforded **16a** (550 mg, 86%) as a yellow solid; $R_f = 0.51$ (2:98 MeOH:EtOAc); mp = 118-119°C; ^{1}H NMR ($CDCl_3$): δ 8.10 (m, 2H), 7.77 (m, 2H), 7.03 (t, 1H), 5.05 (m, 2H, $J_{P\text{-}H}$ = 7.0 Hz), 3.55 (m, 8H), 2.98 (bs, 2H); ^{31}P NMR ($CDCl_3$): δ 16.18; IR (Nujol): 1664, 1627, 1591 cm^{-1}. Anal. Calcd. for $C_{15}H_{17}Br_2N_2O_4P$: C, 37.53; H, 3.57; N, 5.83. Found: C, 37.80; H, 3.57; N, 5.56.

The intermediate compound **19** was prepared as follows.

a. bis(2-Bromoethyl)amine hydrobromide (**18Br**)

$$HN(CH_2CH_2OH)_2 \xrightarrow[\Delta]{48\%\ HBr} \underset{\mathbf{18Br}}{HBr\text{-}HN(CH_2CH_2Br)_2}$$

Hydrobromic acid (75 mL, 0.45 mol, 48% by wt) was added slowly, with stirring, to diethanolamine (11.0 g, 0.10 mol) at 0°C. The reaction mixture was heated to reflux and then distilled through a 14/20 vigreaux column. The distillate temperature was 100°C for the first 15 mL collected and 125°C for the remainder. After a total of 40 mL of distillate was collected, additional 48% HBr (50 mL, 0.30 mol) was added and 50 mL of distillate was collected. Hydrobromic acid (25 mL, 48%) was added and the reaction mixture was refluxed overnight. The reaction mixture was then distilled (~30 mL collected) and the still pot residue was poured into acetone at –78°C. The white solid that precipitated (10.3 g, 22% diethanolamine hydrobromide and 78% product **18Br** as determined by ^{1}H NMR) was collected by filtration. This impure product can either be used directly in the subsequent phosphorylation reaction or can be purified by repeated recrystallization from acetone. This recrystallization procedure actually crystallizes out diethanolamine hydrobromide, so that purer product **18Br** is recovered from the filtrate upon successive recrystallization attempts; <u>^{1}H NMR</u> of **18Br** (D_2O): δ 3.55 (t, 4H), 3.45 (t, 4H).
<u>^{1}H NMR</u> of diethanolamine hydrobromide (D_2O): δ 3.78 (t, 4H), 3.12 (t, 4H).

b. bis(2-Bromoethyl)phosphoramidic dichloride (**19**):

$$POCl_3 + \underset{\mathbf{18Br}}{HBr\text{-}HN(CH_2CH_2Br)_2} \xrightarrow[-40^\circ C\ -\ 0^\circ C]{TEA,\ CH_2Cl_2} \underset{\mathbf{19}}{Cl-\overset{O}{\overset{\|}{\underset{Cl}{\underset{|}{P}}}}-N(CH_2CH_2Br)_2}$$

Phosphorus oxychloride (0.30 mL, 3.2 mmol) was added slowly to a suspension of hydrobromide salt **18Br** (1.00 g, 3.2 mmol) in CH_2Cl_2 (22 mL) at –40°C under

argon. Triethylamine (1.20 mL, 8.6 mmol) was added dropwise via syringe over 5 min while the reaction mixture was vigorously stirred to avoid local heating. The cloudy white reaction mixture was warmed to 0°C over 2.5 h and stirred at 0 °C for 4 h. Saturated ammonium chloride (8 mL) was added and the mixture was extracted with CH_2Cl_2 (3x). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (20:80 EtOAc:hexanes) afforded **19** (0.76 g, 68%) as a white solid; $R_f = 0.56$ (20:80 EtOAc:hexanes); mp = 43 – 44 °C; lit mp = 39 – 40°C[25]; ^{1}H NMR ($CDCl_3$): δ 3.73 (dt, 4H), 3.55 (t, 4H); ^{31}P NMR ($CDCl_3$): δ 16.48.

The intermediate compound **26a** was prepared as follows.

c. 2-(1,4-Dimethoxynaphthyl)methyl *N,N*-bis(2-bromoethyl) phosphorodiamidate (**26a**). Lithium bis(trimethylsilyl)amide (6.05 mL, 6.05 mmol, 1 M in THF) was added dropwise via syringe to a solution of alcohol **23a** (1.20 g, 5.50 mmol) in THF (20 mL) at –78 °C under argon. The resulting solution was stirred for 5 min and then added dropwise to a solution of bis(2-bromoethyl)-phosphoramidic dichloride (**19**) (2.29 g, 6.60 mmol) in THF (50 mL) at –78 °C. The reaction mixture was stirred at –78 °C for 1.5 h and then warmed to –20 °C. Gaseous ammonia was passed through the reaction mixture for 10 min. The mixture was stirred for 10 min, aqueous HCl (2%, 70 mL) was added and the mixture was extracted with EtOAc (3x). The combined organic layers were washed with saturated NaCl (2x), dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (70:30 EtOAc:hexanes) afforded **26a** (1.20 g, 43%) as a white solid; $R_f = 0.33$ (70:30 EtOAc:hexanes); mp = 112 – 113°C; ^{1}H NMR ($CDCl_3$): δ 8.23 (dd, 1H), 8.06 (dd, 1H), 7.54 (m, 2H), 6.84 (s, 1H), 5.26 (m, 2H, $J_{P\text{-}H} = 8.1$ Hz), 4.01 (s, 3H), 3.94 (s, 3H), 3.50 (m, 8H), 2.78 (bs, 2H); ^{31}P NMR ($CDCl_3$): δ 15.67.

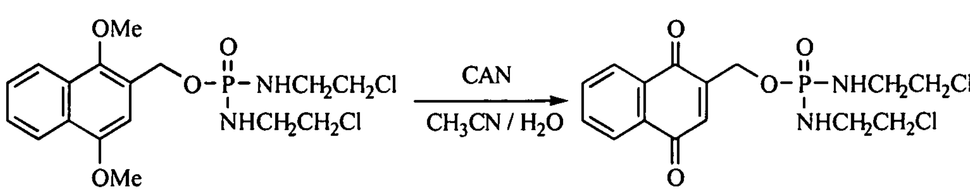
Example 6. 2-(3-Methyl-1,4-naphthoquinonyl)methyl *N,N*-bis(2-bromoethyl) phosphorodiamidate (**16b**)

Compound **16b** was prepared from **23b** (470 mg, 0.897 mmol) as described above for **16a** to give 230 mg (52%) of the product as a yellow solid after column chromatography (6:94 MeOH:$CHCl_3$); $R_f = 0.28$ (6:94 MeOH:$CHCl_3$); mp = 117 – 119°C; ^{1}H NMR ($CDCl_3$): δ 8.12 (m, 2H), 7.76 (m, 2H), 5.06 (m, 2H, $J_{P\text{-}H} = 7.6$ Hz), 3.50 (m, 8H), 3.02 (bs, 2H), 2.34 (s, 3H); ^{31}P NMR ($CDCl_3$): δ 15.72; IR (Nujol): 1662, 1626, 1594, 1568 cm^{-1}; HPLC (50:50 CH_3CN:0.1% TFA / H_2O): 4.78 min, 95.6%; FAB MS: Calcd. for $C_{16}H_{19}Br_2N_2O_4P$: $(M+H)^+$ 492.9527; Found 492.9542.

The intermediate compound **23b** was prepared as follows.

a. 2-(3-Methyl-1,4-dimethoxynaphthyl)methyl *N,N*-bis(2-bromoethyl)phosphorodiamidate (**26b**). Compound **26b** was prepared from **23b** (480 mg, 2.07 mmol) as described above for **26a** to give 480 mg (44%) of the product as a viscous yellow oil after column chromatography (2:98 MeOH:EtOAc); $R_f = 0.63$ (2:98 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$): δ 8.09 (d, 2H), 7.41 (m, 2H), 5.31 (m, 2H, $J_{P\text{-}H} = 7.1$ Hz), 3.98 (s, 3H), 3.89 (s, 3H), 3.48 (m, 8H), 2.77 (bs, 2H), 2.53 (s, 3H); ^{31}P NMR ($CDCl_3$): δ 15.32.

Example 7. 2-(1,4-Naphthoquinonyl)methyl bis[*N*-(2-chloroethyl)] phosphorodiamidate (**17**):



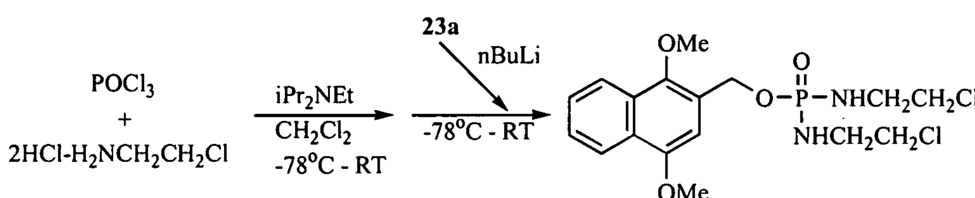
28 **17**

Ceric ammonium nitrate (0.20 g, 0.37 mmol) in H_2O (2 mL) was added in portions over 10 min to a solution of **28** (62.0 mg, 0.15 mmol) in CH_3CN (5

mL). The solution was stirred at room temperature for 1 h. Water (3 mL) was added and the mixture was extracted with $CHCl_3$ (3x). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (78:22 $CHCl_3$:Acetone until less polar impurities are removed, then 67:33 $CHCl_3$:Acetone) afforded **17** (42.4 mg, 74%) as a yellow solid; $R_f = 0.24$ (67:33 $CHCl_3$:Acetone); mp = 109 – 110 °C; ^{1}H NMR ($CDCl_3$): δ 8.10 (m, 2H), 7.77 (m, 2H), 7.03 (t, 1H, J = 1.9 Hz), 5.04 (dd, 2H, J = 1.9 and 6.8 Hz), 3.65 (t, 4H), 3.36 (dt, 4H), 3.20 (bs, 2H); ^{31}P NMR ($CDCl_3$): δ 14.83; IR (Nujol): 1662, 1633, 1594 cm^{-1}. Anal. Calcd. for $C_{15}H_{17}Cl_2N_2O_4P$: C, 46.06; H, 4.38; N, 7.16. Found: C, 46.04; H, 4.08; N, 6.78.

The intermediate compound **28** was prepared as follows.

a. 2-(1,4-Dimethoxynaphthyl)methyl bis[*N*-(2-chloroethyl)] phosphorodiamidate (**28**):

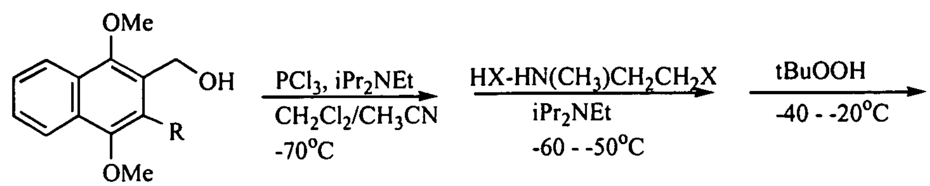


28

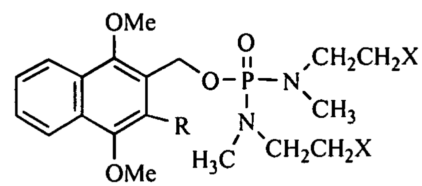
Phosphorus oxychloride (0.043 mL, 0.46 mmol) was added to a suspension of *N*-(2-chloroethyl)amine hydrochloride (112 mg, 0.96 mmol) in CH_2Cl_2 (10 mL) at –78 °C under argon. Diisopropylethylamine (0.34 mL, 1.92 mmol) was added dropwise and the reaction mixture was warmed to room temperature over 2 h and stirred for 7 h. After the phosphorylation reaction was completed, n-butyllithium (0.18 mL, 0.46 mmol, 2.5 M in hexanes) was added to a solution of alcohol **23a** (100 mg, 0.46 mmol) in CH_2Cl_2 (5 mL) in a second flask at –78°C under argon. The resulting solution was stirred for 15 min. The contents of the flask containing the phosphorylating reagent were added via syringe to the second solution with vigorous stirring at –78°C. The reaction mixture was slowly warmed to room temperature, stirred overnight, and then

concentrated under reduced pressure until the volume was reduced to a third. The salts were separated and the crude product was purified by column chromatography (78:22 $CHCl_3$:Acetone until less polar impurities are removed, then 67:33 $CHCl_3$:Acetone) to afford **28** (62.0 mg, 32%, 87% based on recovered **23a**, 63.0 mg) as a light yellow oil; $R_f = 0.31$ (78:22 $CHCl_3$:Acetone); ^1H NMR ($CDCl_3$): δ 8.24 (dd, 1H), 8.06 (dd, 1H), 7.55 (m, 2H), 6.84 (s, 1H), 5.25 (d, 2H, J = 7.9 Hz), 4.01 (s, 3H), 3.94 (s, 3H), 3.59 (t, 4H), 3.28 (dt, 4H), 3.04 (bs, 2H); ^{31}P NMR ($CDCl_3$): δ 14.72.

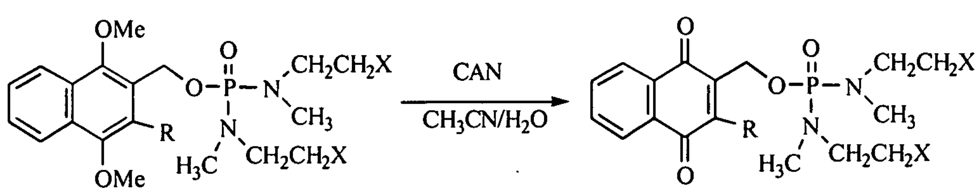
Scheme For Examples 8-10



23a: R = H
23b: R = CH_3



41a: X = Br; R = H
41b: X = Br; R = CH_3
41c: X = Cl; R = H



41a: X = Br; R = H
41b: X = Br; R = CH_3
41c: X = Cl; R = H

40a: X = Br; R = H
40b: X = Br; R = CH_3
40c: X = Cl; R = H

Example 8. 2-(1,4-Naphthoquinonyl)methyl bis[*N*-methyl-*N*-(2-bromoethyl)]phosphorodiamidate (**40a**):

Ceric ammonium nitrate (510 mg, 0.93 mmol) in H_2O (5 mL) was added in portions over 10 min to a solution of **41a** (200 mg, 0.372 mmol) in CH_3CN (17 mL). The solution was stirred at room temperature for 1 h and extracted with $CHCl_3$ (3x). The combined organic layers were washed with H_2O and

saturated NaCl, dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (2:98 MeOH:CH_2Cl_2) afforded **40a** (164 mg, 87%) as a viscous brown oil; $R_f = 0.16$ (2:98 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 8.10 (m, 2H), 7.77 (m, 2H), 7.03 (t, 1H, J = 2.0 Hz), 5.04 (dd, 2H, J = 2.0 and 6.4 Hz), 3.49 (m, 8H), 2.78 (d, 6H, J = 9.7 Hz); ^{31}P NMR ($CDCl_3$): δ 18.00; IR (neat): 1664, 1632, 1595 cm^{-1}.

Anal. Calcd. for $C_{17}H_{21}Br_2N_2O_4P$: C, 40.18; H, 4.17; N, 5.51. Found: C, 40.36; H, 4.09; N, 5.18.

The intermediate compound **41a** was prepared as follows.

a. 2-(1,4-Dimethoxynaphthyl)methyl bis[*N*-methyl-*N*-(2-bromoethyl)] phosphorodiamidate (**41a**). A solution of alcohol **23a** (100 mg, 0.46 mmol) in CH_2Cl_2 (2 mL) and CH_3CN (2 mL) was cooled to –70°C and stirred under argon. Phosphorus trichloride (0.23 mL, 0.46 mmol, 2 M in CH_2Cl_2) was added dropwise followed by the dropwise addition of diisopropylethylamine (0.08 mL, 0.46 mmol). The reaction mixture was stirred for 25 min at –70°C. A solution of *N*-methyl-*N*-(2-bromoethyl)amine hydrobromide (200 mg, 0.92 mmol) in CH_3CN (2.5 mL) was added slowly via syringe, followed by additional diisopropylethylamine (0.32 mL, 1.84 mmol). The temperature was raised to –60 to –50°C and the reaction was stirred for 1.25 h. t-Butyl hydroperoxide (1.1 mL, 5-6 M in decane) was added and the reaction mixture was warmed to –40 to –20°C and stirred for 30 min. Water (3 mL) was added and the mixture was warmed to room temperature and extracted with CH_2Cl_2 (4x). The combined organic layers were washed with H_2O and saturated NaCl, dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (2:98 MeOH:EtOAc) afforded **41a** (190 mg, 77%) as a light yellow oil; $R_f = 0.33$ (2:98 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$): δ 8.24 (dd, 1H), 8.06 (dd, 1H), 7.54 (m, 2H), 6.87 (s, 1H), 5.24 (d, 2H, J = 7.4 Hz), 4.01 (s, 3H), 3.93 (s, 3H), 3.43 (m, 8H), 2.71 (d, 6H, J = 9.7 Hz); ^{31}P NMR ($CDCl_3$): δ 17.60.

Example 9. 2-(3-Methyl-1,4-naphthoquinonyl)methyl bis[*N*-methyl-*N*-(2-bromoethyl)] phosphorodiamidate (**40b**):

Compound **40b** was prepared from **41b** (130 mg, 0.235 mmol) as described above for **40a** to give 99 mg (81%) of the product as a yellow oil after column chromatography (3:97 MeOH:CH_2Cl_2); $R_f = 0.20$ (3:97 MeOH:CH_2Cl_2); ^{1}H NMR ($CDCl_3$): δ 8.12 (m, 2H), 7.75 (m, 2H), 5.04 (d, 2H, J = 6.4 Hz), 3.43 (m, 8H), 2.71 (d, 6H, J = 9.7 Hz), 2.35 (s, 3H); ^{31}P NMR ($CDCl_3$): δ 17.77; IR (neat): 1663, 1626, 1595 cm^{-1}. Anal. Calcd. for $C_{18}H_{23}Br_2N_2O_4P$: C, 41.40; H, 4.44; N, 5.36. Found: C, 41.31; H, 4.28; N, 5.13.

The intermediate compound **41b** was prepared as follows.

a. 2-(3-Methyl-1,4-dimethoxynaphthyl)methyl bis[*N*-methyl-*N*-(2-bromoethyl)] phosphorodiamidate (**41b**). Compound **41b** was prepared from alcohol **23b** (100 mg, 0.431 mmol) and *N*-methyl-*N*-(2-bromoethyl)amine hydrobromide as described above for **41a** to give 135 mg (57%) of the product as a yellow oil after column chromatography (2:98 MeOH:EtOAc); $R_f = 0.37$ (2:98 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$): δ 8.09 (dd, 2H), 7.52 (m, 2H), 5.28 (d, 2H, J = 5.8 Hz), 3.97 (s, 3H), 3.89 (s, 3H), 3.41 (m, 8H), 2.67 (d, 6H, J = 9.7 Hz), 2.53 (s, 3H); ^{31}P NMR ($CDCl_3$): δ 17.28.

Example 10. 2-(1,4-Naphthoquinonyl)methyl bis[*N*-methyl-*N*-(2-chloroethyl)]phosphorodiamidate (**40c**).

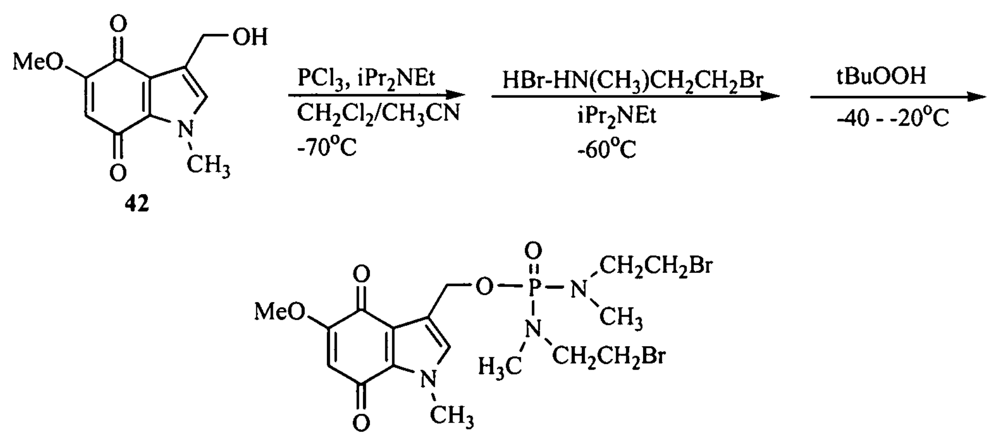
Compound **40c** was prepared from **41c** (130 mg, 0.289 mmol) as described above for **40a** to give 105 mg (87%) of the product as a brown oil after column chromatography (2:98 MeOH:EtOAc); $R_f = 0.55$ (2:98 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$): δ 8.10 (m, 2H), 7.77 (m, 2H), 7.03 (t, 1H, J = 2.0), 5.03 (dd, 2H, J = 2.0 and 6.4 Hz), 3.66 (t, 4H), 3.42 (m, 4H), 2.78 (d, 6H, J = 9.9 Hz); ^{31}P NMR ($CDCl_3$): δ 18.21 ppm; IR (neat): 1664, 1633, 1595 cm^{-1}. Anal. Calcd.

for $C_{17}H_{21}Cl_2N_2O_4P$: C, 48.70; H, 5.05; N, 6.68. Found: C, 48.36; H, 5.02; N, 6.35.

The intermediate compound **41c** was prepared as follows.

a. 2-(1,4-Dimethoxynaphthyl)methyl bis[*N*-methyl-*N*-(2-chloroethyl)]phosphorodiamidate (**41c**). Compound **41c** was prepared from alcohol **23a** (100 mg, 0.458 mmol) and *N*-methyl-*N*-(2-chloroethyl)amine hydrochloride as described above for **41a** to give 132 mg (64%) of the product as a light yellow oil after column chromatography (2:98 MeOH:EtOAc); $R_f = 0.33$ (2:98 MeOH:EtOAc); ^{1}H NMR ($CDCl_3$): δ 8.24 (dd, 1H), 8.06 (dd, 1H), 7.54 (m, 2H), 6.88 (s, 1H), 5.24 (d, 2H, J = 7.3 Hz), 4.00 (s, 3H), 3.93 (s, 3H), 3.63 (t, 4H), 3.37 (dt, 4H), 2.72 (d, 6H, J = 9.6 Hz); ^{31}P NMR ($CDCl_3$): δ 17.91.

Example 11. 3-(5-Methoxy-1-methyl-4,7-indolequinonyl)-methyl bis[*N*-methyl-*N*-(2-bromoethyl)] phosphorodiamidate (**47**):



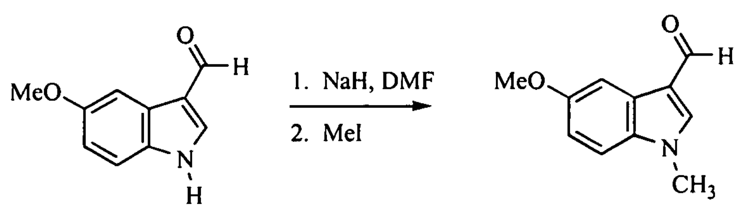
47

A solution of alcohol **42** (50 mg, 0.23 mmol) in CH_2Cl_2 (3 mL) and CH_3CN (2 mL) was cooled to –70°C and stirred under argon. Phosphorus trichloride (0.115 mL, 0.23 mmol, 2 M in CH_2Cl_2) was added dropwise followed by the dropwise addition of diisopropylethylamine (0.04 mL, 0.23 mmol). The

solution was stirred for 15 min and *N*-methyl-*N*-(2-bromoethyl)amine hydrobromide (99 mg, 0.46 mmol) in CH_3CN (1.5 mL) was added slowly via syringe followed by the dropwise addition of diisopropylethylamine (0.16 mL, 0.92 mmol). The mixture was stirred for 2 h below –60°C. t-Butyl hydroperoxide (0.23 mL, 5-6 M in decane) was added and the solution was warmed to –40 to –20°C and stirred for 1 hr. Water (3 mL) was added and the mixture was extracted with CH_2Cl_2 (4x). The combined organic layers were washed with H_2O and saturated NaCl, dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (10:90 EtOH:ether) afforded **47** (25 mg, 20%) as an orange oil; $R_f = 0.48$ (10:90 EtOH:ether); ^{1}H NMR ($CDCl_3$): □ 6.91 (s, 1H), 5.68 (s, 1H), 5.14 (d, 2H, J = 7.5 Hz), 3.96 (s, 3H), 3.83 (s, 3H), 3.42 (m, 8H), 2.72 (d, 6H, J = 9.7 Hz); ^{31}P NMR ($CDCl_3$): □ 16.51; IR (neat): 1673, 1643, 1597, 1511 cm^{-1}; HPLC (40:60 CH_3CN:0.1% TFA / H_2O): 8.78 min, 95.9%; FAB MS: Calcd. for $C_{17}H_{24}Br_2N_3O_5P$: $(M+H)^+$ 539.9900; Found: 539.9901.

The intermediate compound **42** was prepared as follows.

a. 5-Methoxy-1-methylindole-3-carboxaldehyde (**43**)

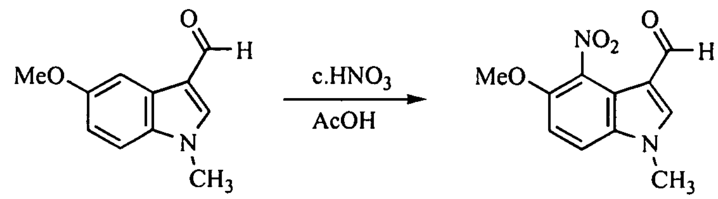


43

5-Methoxyindole-3-carboxaldehyde (300 mg, 1.71 mmol) was added in portions over 5 min to a suspension of sodium hydride (82 mg, 2.05 mmol, 60% dispersion in mineral oil) in DMF (8 mL) stirring under argon. The mixture was stirred for 30 min, methyl iodide (0.13 mL, 2.05 mmol) was added and the mixture was stirred for 1 h. Sodium bicarbonate (10%, 40 mL) was added and the mixture was extracted with EtOAc (4x). The combined organic layers were washed with sodium bicarbonate (10%, 2x) and saturated NaCl, dried ($MgSO_4$),

filtered and evaporated. Column chromatography of the crude product (50:50 EtOAc:hexanes) afforded **43** (320 mg, 99%) as a light yellow solid; $R_f = 0.35$ (50:50 EtOAc:hexanes); mp = 130 – 132 °C; lit mp = 132 – 133°C[53]; ^{1}H NMR ($CDCl_3$): δ 9.95 (s, 1H), 7.79 (d, 1H, J = 2.4 Hz), 7.62 (s, 1H), 7.25 (d, J = 8.8 Hz), 6.96 (dd, 1H, J = 2.4 and 8.9 Hz), 3.90 (s, 3H), 3.85 (s, 3H).

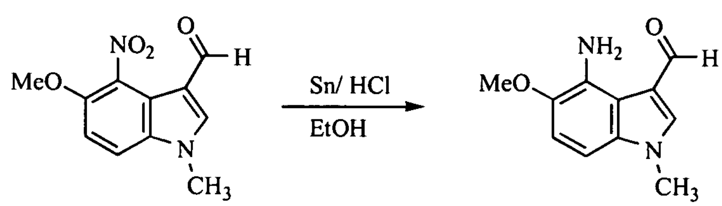
b. 5-Methoxy-1-methyl-4-nitroindole-3-carboxaldehyde (**44**)



43 **44**

A mixture of concentrated HNO_3 (2 mL) in AcOH (11 mL) was added dropwise over 40 min to a solution of **43** (1.08 g, 5.71 mmol) in AcOH (70 mL) at 0°C. The mixture was warmed to room temperature, stirred overnight, and poured over ice. The product (**44**) was collected by filtration, washed with H_2O, and dried to give 1.18 g (88%) of a yellow solid; $R_f = 0.49$ (EtOAc); mp = 195 – 197°C; lit mp = 197 – 198°C[53]; ^{1}H NMR ($CDCl_3$): δ 9.84 (s, 1H), 7.83 (s, 1H), 7.45 (d, 1H, J = 9.0 Hz), 7.11 (d, 1H, J= 9.1 Hz), 3.96 (s, 3H), 3.90 (s, 3H).

c. 4-amino-5-methoxy-1methylindole-3-carboxaldehyde (**45**)

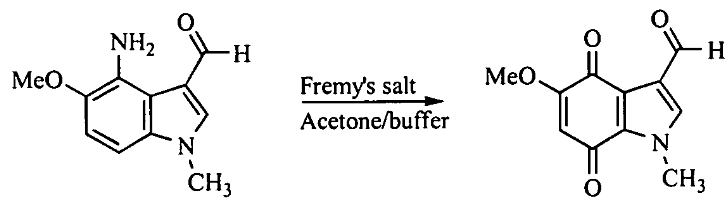


44 **45**

Tin powder (2.65 g, 22.3 mmol) was added to a suspension of nitroindole **44** (600 mg, 2.56 mmol) in EtOH (90 mL). Hydrochloric acid (3 M, 36 mL) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was decanted from the excess tin and added in portions to saturated

sodium bicarbonate (200 mL). The mixture was extracted with EtOAc (4x) and the combined extracts were washed with sodium bicarbonate (1 M, 3x) and saturated NaCl (2x), dried ($MgSO_4$), filtered and evaporated. Compound **45** was isolated as a dark yellow oil (513 mg, 98%) and used in the following reaction without further purification; R_f = 0.67 (EtOAc); ^{1}H NMR ($CDCl_3$): δ 9.61 (s, 1H), 7.55 (s, 1H), 6.94 (d, 1H, J = 8.6 Hz), 6.53 (d, 1H, J= 8.6 Hz), 5.79 (bs, 2H), 3.88 (s, 3H), 3.76 (s, 3H).

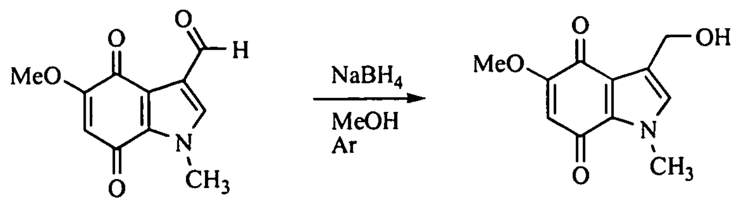
d. 3-Formyl-5-methoxy-1-methylindole-4,7-dione (**46**)



45 **46**

Potassium nitrosodisulfonate (1.85 g, 6.89 mmol) in H_2O (19 mL) was added to a solution of amine **45** (352 mg, 1.72 mmol) in acetone (37 mL). Sodium phosphate buffer (0.4 M, pH 6, 10 mL) was added and the reaction mixture was stirred at room temperature for 1.5 h. The acetone was removed under reduced pressure and the yellow solid was collected by filtration and washed with H_2O. The solid was taken up in warm EtOAc (100 mL) and a small amount of an insoluble solid was removed by filtration. The filtrate was rotovaped and the product was passed through a plug of silica gel (100:10:0.5 $CHCl_3$:EtOAc:MeOH) to afford **46** (310 mg, 82%) as a yellow solid; R_f = 0.44 (100:10:0.5 $CHCl_3$:EtOAc:MeOH); ^{1}H NMR ($CDCl_3$): δ 10.41 (s, 1H), 7.45 (s, 1H), 5.77 (s, 1H), 4.03 (s, 3H), 3.87 (s, 3H).

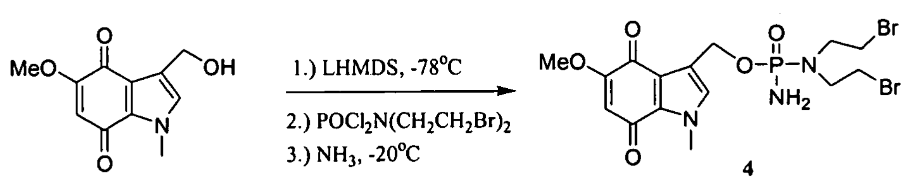
e. 3-Hydroxymethyl-5-methoxy-1-methylindole-4,7-dione (**42**)



46 **42**

Aldehyde **46** (50 mg, 0.228 mmol) was added to anhydrous MeOH (30 mL) that had been degassed by bubbling with argon for 1.5 h. The suspension was degassed with argon for 15 min, $NaBH_4$ (65 mg, 1.71 mmol) was added and the reaction mixture was stirred for 2 h. A persistent light yellow color indicated that the solvent was thoroughly degassed and the hydroquinone had formed. The solution turned dark orange following air-oxidation to the quinone. The MeOH was removed under reduced pressure and the residue was taken up in CH_2Cl_2 (30 mL), washed with H_2O (2x) and saturated NaCl, dried ($MgSO_4$), filtered and evaporated. Column chromatography of the crude product (EtOAc) afforded **42** (24.2 mg, 48%) as a bright orange solid; $R_f = 0.57$ (EtOAc); mp = 182 – 184°C; lit. mp = 185 – 186°C[53]; ^{1}H NMR (CDCl$_3$): δ 6.71 (s, 1H), 5.69 (s, 1H), 4.64 (s, 2H), 3.94 (s, 3H), 3.85 (s, 3H).

Example 12. 3-(5-Methoxy-1-methyl-4,7-indolequinonyl)methyl N,N-bis(2-bromoethyl)phosphorodiamidate (**4**):



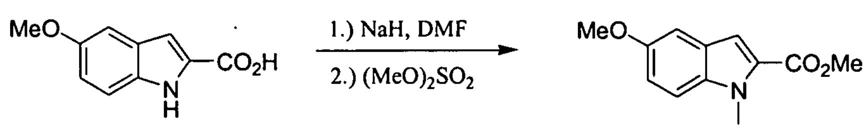
4

LHMDS (0.25mL, 1.0M in THF) was added dropwise to a solution of 3-hydroxymethyl-5-methoxy-1-methylindole-4,7-dione **12** (50mg, 0.23 mmol) in THF (10mL). The solution was stirred for 10 min at –78 °C. A solution of $Cl_2P(O)N(CH_2CH_2Br)_2$ (87mg, 0.25 mmol) in THF (5mL) was added all at once

to the alkoxide, and the resulting solution was stirred for 1.5 h at -78 °C. The solution was warmed to –20 °C and ammonia gas was bubbled through the reaction mixture for 6 min. The mixture was stirred for an additional 7 min and then added to CH_2Cl_2 / H_2O and extracted (CH_2Cl_2, 3×). The combined organic layers were dried over Na_2SO_4 and evaporated. Column chromatography of the crude product (EtOAc/Acetone) afforded **4** (17.8mg, 30% based on recovered starting material) as a yellow solid; R_f = 0.47 (50% EtOAc/Acetone); ^{1}H NMR ($CDCl_3$): δ 6.88 (1H, s), 5.71 (1H, s), 5.04 (2H, m), 3.96 (3H, s), 3.84 (3H, s), 3.47 (8H, m), 3.22 (2H, bs); ^{31}P NMR ($CDCl_3$): δ -9.99 (Ref = TPPO); HPLC (40% CH_3CN/0.1% TFA H_2O): 6.97 min.

The intermediate compound **12** was prepared as follows.

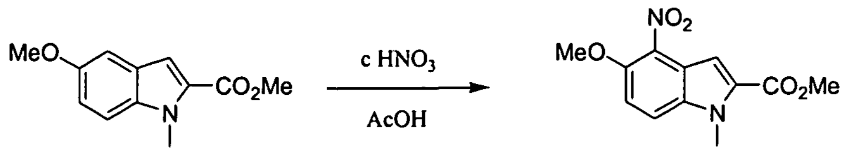
a. 5-Methoxy-1-methylindole-2-carboxylic acid methyl ester (**8**):



8

A solution of 5-methoxyindole-2-carboxylic acid (2.00g, 10.46 mmol) in anhydrous DMF (28 mL) was added to sodium hydride (1.68g, 41.84 mmol, 60% dispersion in mineral oil) at 0°C, under an argon atmosphere. The mixture was stirred for 5 min, dimethyl sulfate (2.96 mL, 31.38 mmol) was added, and the reaction was stirred at room temperature for 48 h. HCl (2M) was added and the resulting mixture extracted with CH_2Cl_2 (3×). The combined organic layers were dried over Na_2SO_4 and evaporated. Column chromatography of the crude product (25% EtOAc/Hexanes) afforded **8** (1.77 g, 78%) as an ivory solid; R_f = 0.84 (25% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$): δ 7.33 (1H, s), 7.30 (1H, d), 7.20 (1H, s), 7.14 (1H,d), 4.05 (3H, s), 3.94 (3H, s), 3.90 (3H, s).

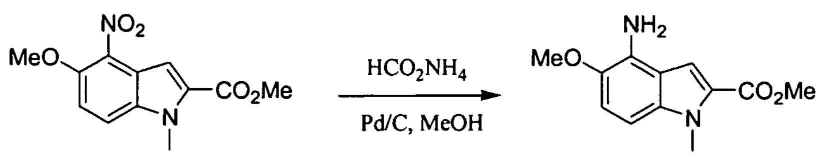
b. 5-Methoxy-1-methyl-4-nitroindole-2-carboxylic acid methyl ester (9)



8 9

A solution of concentrated HNO_3 (2 mL) in AcOH (9 mL) was added to a solution of **8** (830mg, 3.79 mmol) in AcOH (54 mL) at 0 °C. Following the addition, the reaction mixture was warmed to room temperature and stirred for 2 h. The reaction mixture was poured over ice, filtered and the precipitate washed with H_2O. The precipitate was dissolved in CH_2Cl_2 and filtered through a short column of silica gel to afford **9** (780mg, 78%) as a yellow solid; $R_f = 0.53$ (50% EtOAc/hexanes); ^{1}H NMR (CDCl$_3$): δ 7.58 (1H, d), 7.55 (1H, s), 7.18 (1H, d), 4.11 (3H, s), 4.02 (3H, s), 3.94 (3H, s).

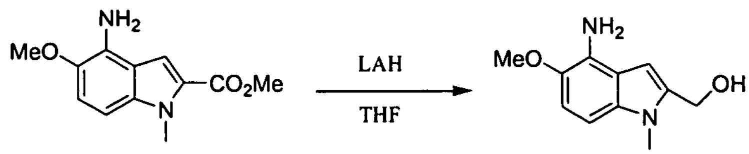
c. 4-Amino-5-methoxy-1-methylindole-2-carboxylic acid methyl ester (**10**):



9 10

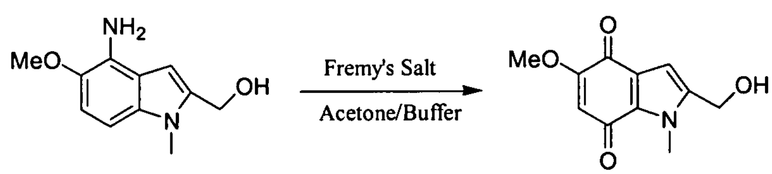
To a solution of **9** (1.0g, 3.78 mmol) in anhydrous methanol (90mL) was added 10%Pd/C (150mg) suspended in anhydrous methanol (15mL) followed by ammonium formate (1.10g, 17.4 mmol). The reaction mixture was stirred for 1 h, filtered through celite and the methanol removed. The residue was taken up in CH_2Cl_2/H_2O and extracted with CH_2Cl_2 (3×). The combined organic layers were dried over Na_2SO_4 and evaporated to afford **10** (595mg, 67%) as a brown solid which was used without further purification; ^{1}H NMR (CDCl$_3$): δ 7.21 (1H, s), 7.06 (1H, d), 6.72 (1H, d), 4.01 (3H, s), 3.90 (3H, s), 3.88 (3H, s).

d. 4-Amino-2-hydroxymethyl-5-methoxy-1-methylindole (**11**)



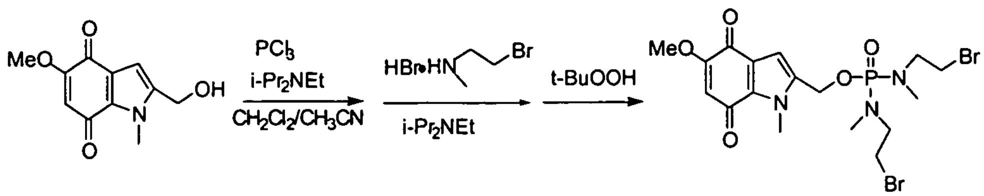
Lithium aluminum hydride (5.84mL, 5.84 mmol, 1.0 M solution in Et_2O) was added to a solution of **10** (595mg, 2.59 mmol) in THF (12mL) under argon. The reaction was then heated at reflux for 15 min, quenched by careful addition of H_2O followed by 1 M NaOH, then extracted with CH_2Cl_2 (3×). The combined organic layers were dried over Na_2SO_4 and evaporated to afford **11** (490mg, 94%) as a brown oil which was used without further purification; [1]H NMR ($CDCl_3$): δ 6.95 (1H, d), 6.71 (1H, d), 6.34 (1H, s), 4.77 (2H, s), 3.87 (3H, s), 3.74 (3H, s).

e. 2-Hydroxymethyl-5-methoxy-1-methylindole-4,7-dione (**12**)



A solution of potassium nitrosodisulfonate $(KSO_3)_2NO$ (590mg, 2.2 mmol) in sodium phosphate buffer (0.4 M, pH = 6, 13mL) was added to a solution of **11** (130mg, 0.63 mmol) in acetone (8mL). The reaction mixture was stirred for 1 h at room temperature, ethyl acetate and water were added, and the aqueous layer was extracted (EtOAc, 3×). The combined organic layers were dried over Na_2SO_4 and evaporated. Column chromatography of the crude product (EtOAc) afforded **12** (122mg, 87%) as an orange solid; R_f = 0.62 (EtOAc); [1]H NMR ($CDCl_3$): δ 6.58 (1H, s), 5.67 (1H, s), 4.68 (2H, s), 4.03 (3H, s), 3.83 (3H, s).

Example 13. 2-(5-Methoxy-1-methyl-4,7-indolequinonyl)methyl bis[N-methyl-



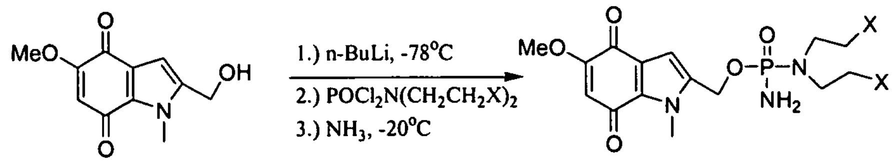
12

5

N-(2-bromoethyl)]phosphorodiamidate (**5**):

PCl_3 (0.12ml, 2.0 M in CH_2Cl_2) was added dropwise to a solution of **12** (50mg, 0.23mmol) in CH_2Cl_2 (3mL) and CH_3CN (2mL) at -78 °C, followed by the dropwise addition of i-Pr_2NEt (0.04mL, 0.23mmol). The reaction was stirred under argon at –78 °C. After 15 min, methylbromoethylamine hydrobromide (99mg, 0.46mmol) in CH_3CN (1.5mL) was added, followed by the dropwise addition of i-Pr_2NEt (0.16mL, 0.92mmol). The mixture was stirred for 2 h, t-BuOOH (0.12 mL, 5-6M in decane) was added, and the reaction mixture was warmed to -20°C and stirred for 1 h. Water (3mL) was added and the mixture was extracted with CH_2Cl_2 (3×). The combined organic extracts were washed with water, dried over Na_2SO_4, and concentrated. Reverse phase column column chromatography (45% MeOH/H_2O) afforded **5** (2.4mg, 2%) as a yellow solid; R_f = 0.33 (5% MeOH/EtOAc); ^{1}H NMR ($CDCl_3$): δ 6.69 (1H, s), 5.71 (1H, s), 5.03 (2H, d), 4.03 (3H, s), 3.84 (3H, s), 3.40 (8H, m), 2.70 (3H, s), 2.66 (3H, s); ^{31}P NMR ($CDCl_3$): δ -10.01 (Ref = TPPO); HPLC (40% CH_3CN/0.1% TFA H_2O): 12.73 min; FAB MS: Calcd. for : 539.9898 found 539.9904.

Scheme for Examples 14 and 15



12

6a: X = Cl
6b: X = Br

Example 14. 2-(5-Methoxy-1-methyl-4,7-indolequinonyl)methyl N,N-bis(2-chloroethyl)phosphorodiamidate (**6a**):

n-Butyllithium (0.05mL, 2.5M in hexanes) was added dropwise to a solution of **12** (25mg, 0.11 mmol) in THF (3mL. The solution was allowed to stir for 10 min at –78 °C, and a solution of $Cl_2P(O)N(CH_2CH_2Cl)_2$ (34mg, 0.13mmol) in THF (0.5mL) was added all at once. The solution was stirred for 1.5 h at –78 °C then warmed to -20°C and ammonia gas was bubbled through the reaction mixture for 7 min. The reaction was stirred for an additional 8 min and then added to CH_2Cl_2 / H_2O and extracted (3×). The combined organic layers were dried over Na_2SO_4 and evaporated. Column chromatography of the crude product (20% EtOAc/Acetone) afforded **6a** (27.6mg, 59%) as a golden solid; R_f = 0.47 (20% EtOAc/Acetone); ^{1}H NMR ($CDCl_3$): δ 6.68 (1H, s), 5.69 (1H, s), 5.01 (2H, m), 4.02 (3H, s), 3.83 (3H, s), 3.65 (4H, m), 3.47 (4H, m), 2.03 (2H, bs); ^{31}P NMR ($CDCl_3$): δ -9.35 (Ref = TPPO); HPLC (40% CH_3CN/0.1% TFA H_2O): 5.93 min; FAB MS: Calcd. for : 446.0415 found 446.0419.

Example 15. 2-(5-Methoxy-1-methyl-4,7-indolequinonyl)methyl N,N-bis(2-bromoethyl)phosphorodiamidate (**6b**)

The title compound was prepared on a 0.11 mmol scale as described above for **6a**, except that $Cl_2P(O)N(CH_2CH_2Br)_2$ was used as the phosphorylating agent. Column chromatography of the crude product (30% EtOAc/Acetone) afforded **6b** (33.8mg, 60%) as a peach solid; R_f = 0.53 (30% EtOAc/Acetone); ^{1}H NMR ($CDCl_3$): δ 6.69 (1H, s), 5.70 (1H, s), 5.05 (2H, d), 4.02 (3H, s), 3.83 (3H, s), 3.473 (8H, m), 2.90 (2H, bs); ^{31}P NMR ($CDCl_3$): δ -

9.66 (Ref = TPPO); HPLC (40% CH_3CN/0.1% TFA H_2O): 7.02 min; FAB MS: Calcd. for : 511.9585 found 511.9579. Anal. Calcd. for $C_{15}H_{20}Br_2N_3O_5P$: C 35.11 H 3.93 N 8.19 found C 35.33 H 4.02 N 7.82.

Example 16. The following illustrate representative pharmaceutical dosage forms, containing a compound of formula I ('Compound X'), for therapeutic or prophylactic use in humans.

(i) Tablet 1	mg/tablet
'Compound X'	100.0
Lactose	77.5
Povidone	15.0
Croscarmellose sodium	12.0
Microcrystalline cellulose	92.5
Magnesium stearate	3.0
	300.0

(ii) Tablet 2	mg/tablet
'Compound X'	20.0
Microcrystalline cellulose	410.0
Starch	50.0
Sodium starch glycolate	15.0
Magnesium stearate	5.0
	500.0

(iii) Capsule	mg/capsule
'Compound X'	10.0
Colloidal silicon dioxide	1.5
Lactose	465.5
Pregelatinized starch	120.0
Magnesium stearate	3.0
	600.0

(iv) Injection 1 (1 mg/ml)	mg/ml
'Compound X' (free acid form)	1.0
Dibasic sodium phosphate	12.0
Monobasic sodium phosphate0.7	
Sodium chloride	4.5
1.0 N Sodium hydroxide solution	
(pH adjustment to 7.0-7.5)	q.s.
Water for injection	q.s. ad 1 mL

(v) Injection 2 (10 mg/ml)	mg/ml
'Compound X' (free acid form)	10.0
Monobasic sodium phosphate0.3	
Dibasic sodium phosphate	1.1
Polyethylene glycol 400	200.0
01 N Sodium hydroxide solution	
(pH adjustment to 7.0-7.5)	q.s.
Water for injection	q.s. ad 1 mL

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art.

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.